Fred Hutch
Cancer Center

CANCER GENOMICS

Lecture 1:
Introduction to Cancer Genome Analysis

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1 Introduction to Cancer Genome Analysis

2 Probabilistic Methods for Mutation Detection

3 Probabilistic Methods for Profiling Copy Number Alteration

4 Additional Topics: Tumor Heterogeneity, Mutation Detection Power, Structural Variation

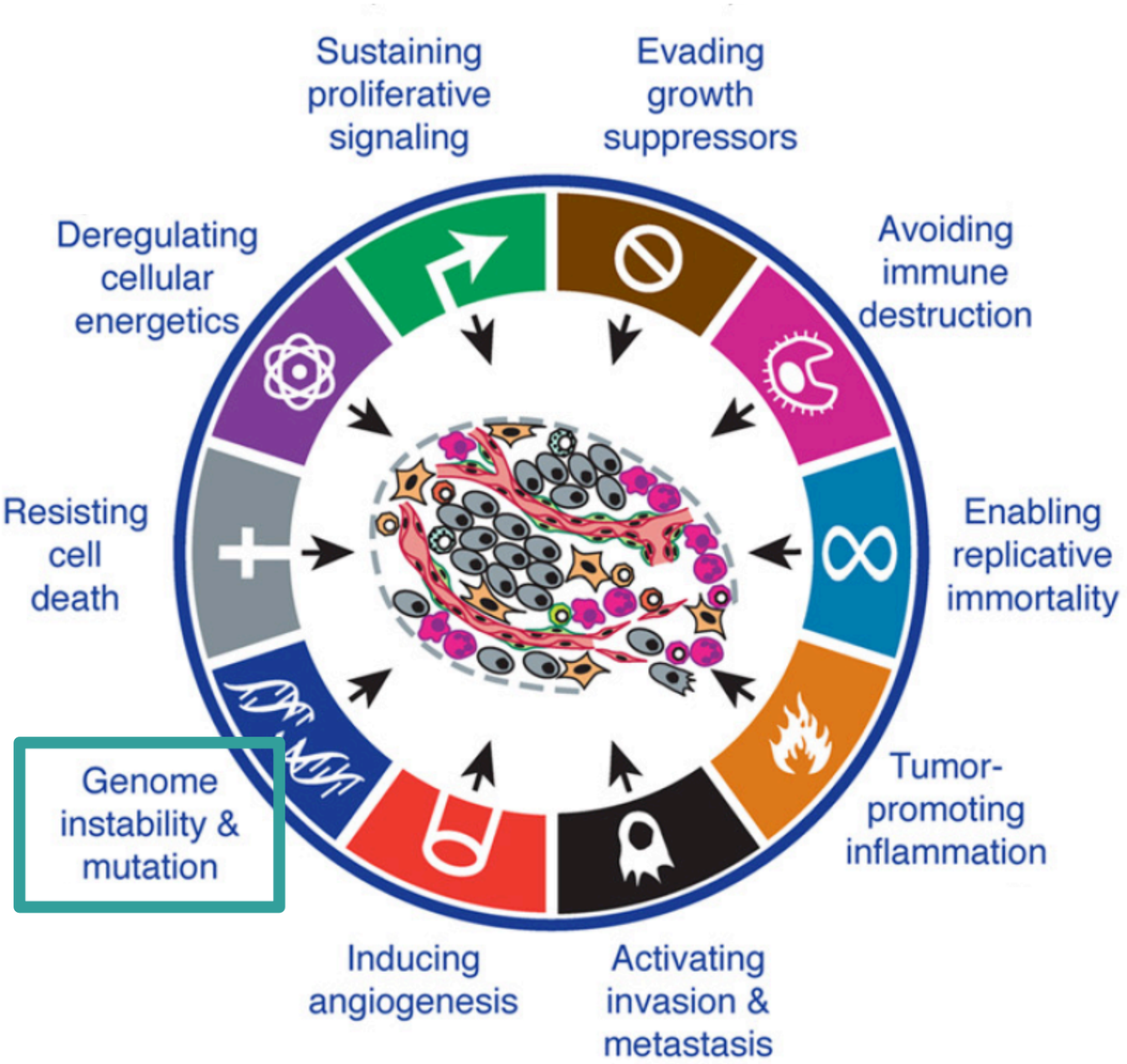
Outline: Introduction to Cancer Genome Analysis

1. **Intro to Cancer Genome Alterations**
   - Genomic alterations in cancer: drivers vs passengers, somatic vs germline
   - Tumor evolution and heterogeneity
2. **Overview of Cancer Genome Analysis**
   - Computational strategy and workflow
   - Tumor DNA Sequencing
   - Types of genomic alterations predicted from tumor sequencing
   - Methods/tools/algorithms in following lectures
3. **Primer on statistical modeling**
   - Binomial probability distribution, Bayesian statistics, parameter learning

The hallmarks of cancer

- All cancers exhibit many of these hallmarks that lead to tumor growth

- **Genome instability** & **mutation** is an enabling characteristic that can result in multiple hallmarks

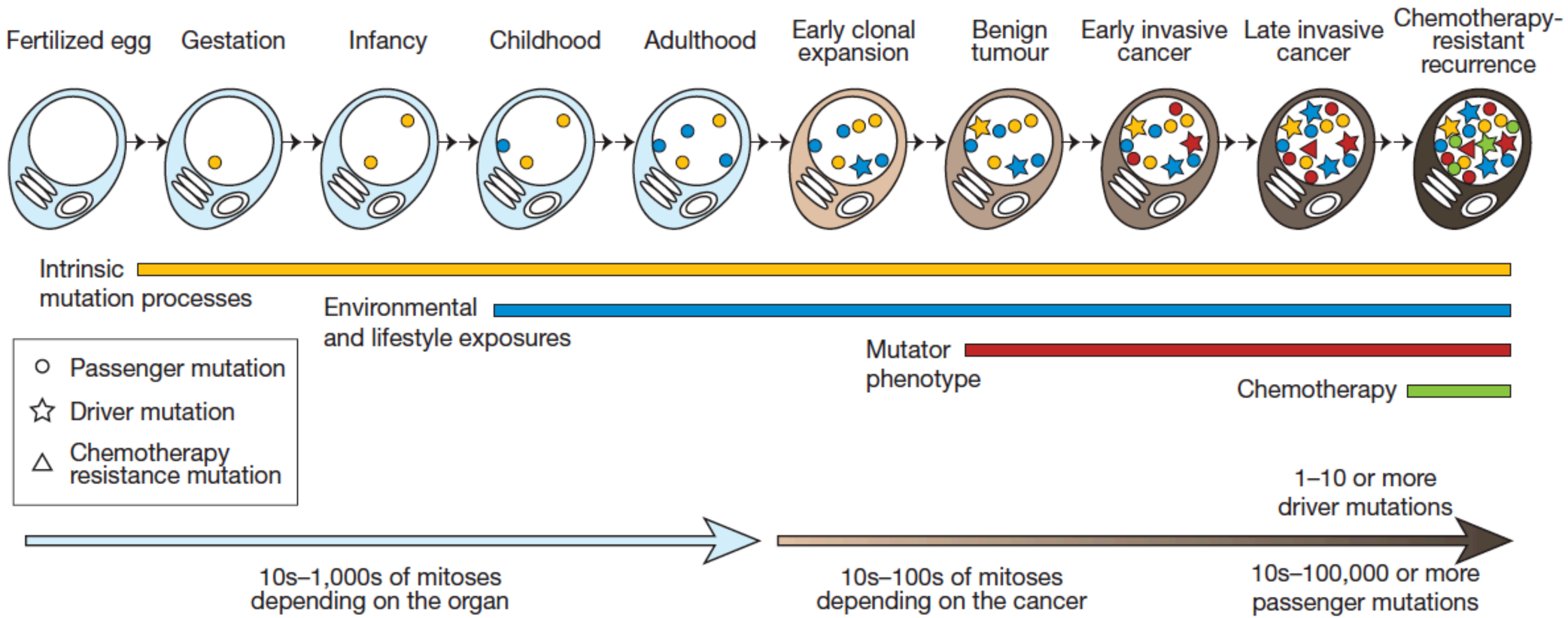


Hannahan & Weinberg. *Cell* **144**:646-74 (2011)

Cancer is a disease of the genome

Cancer progression results from **mutations** acquired throughout lifetime

- Few **driver** mutations, many **passenger** mutations
- Mutational process can be intrinsic and from environmental mutagens



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Stratton, Campbell & Futreal. *Nature* **458**:719-24 (2009)

Genomic Variation: Somatic and Germline

Variant or Mutation or Alteration or Polymorphism

- Changes in the genome sequence of a sample compared to a reference sequence

Germline Variant

- Chromosomes: 22 autosomal pairs + 1 sex pair
  - Each set inherited from maternal and paternal germline cells
- Variant inherited from one or both parental chromosomes
- Source of genetic differences between ancestral populations and individuals
- Polymorphism: >1% frequency in a population

Somatic Variant

- Mutation acquired during individual's lifetime
- Important to identify in sporadic cancers and other non-familial diseases

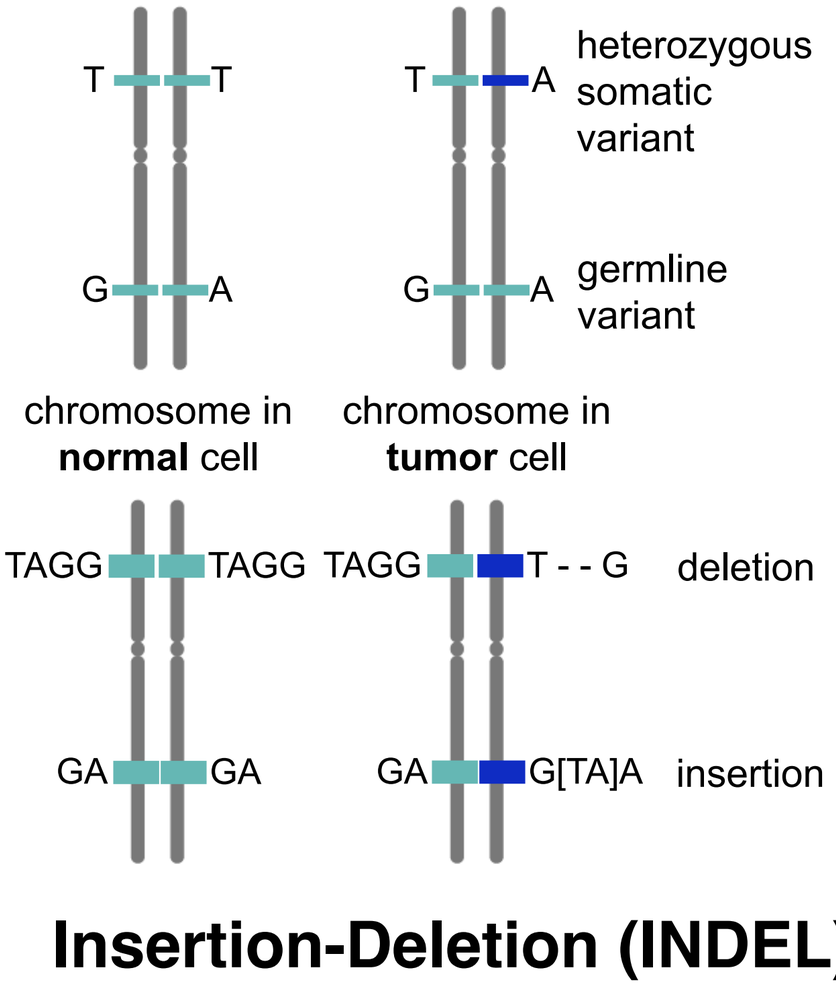
Types of Genomic Variation: Small/Short mutations

1. Single nucleotide base substitutions

- Germline single nucleotide polymorphism (SNP)
- Somatic single nucleotide variant (SNV)

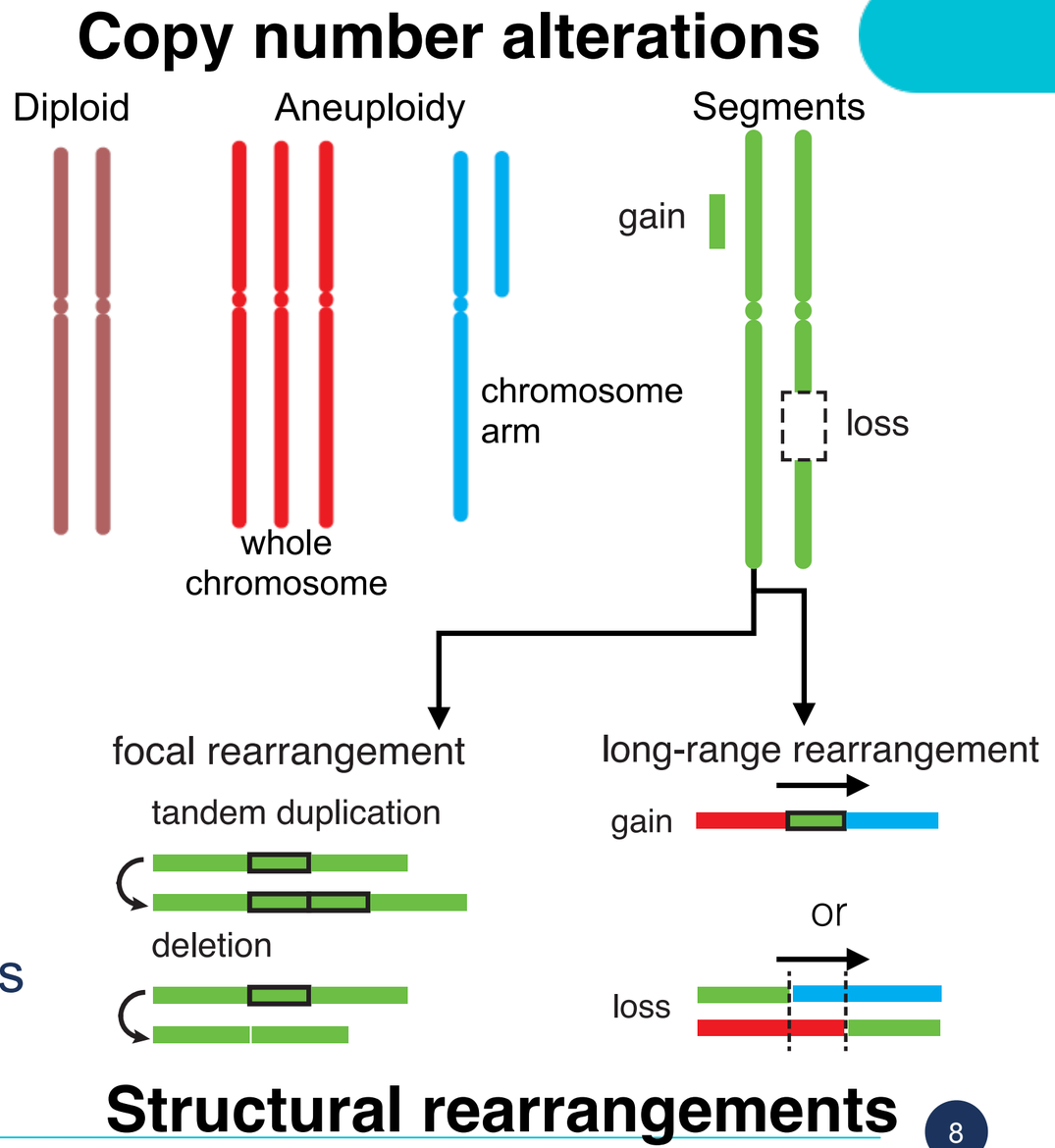
2. Small insertions or deletions

- Germline or somatic insertion or deletion (INDEL)
- Small indels: 1 bp - 20 bps
- Large indels: 20 - 10,000 bps



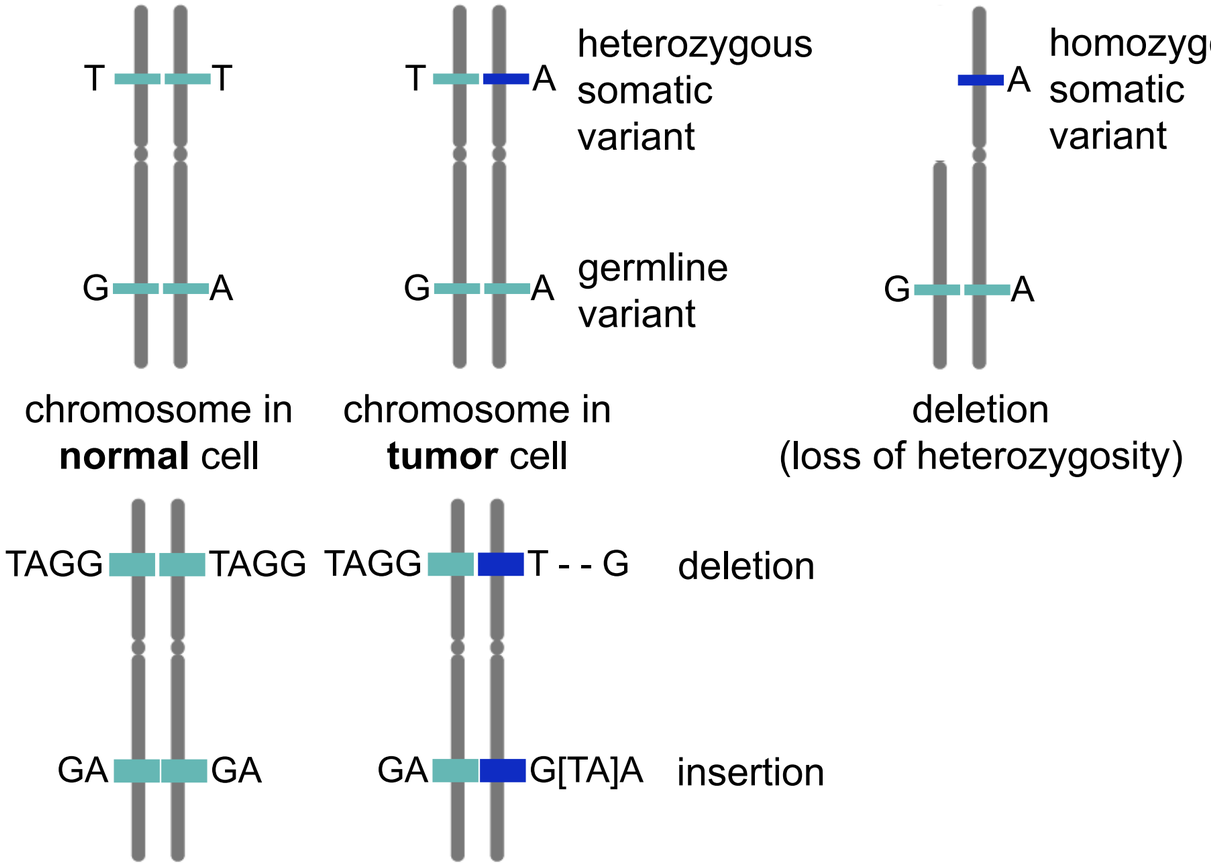
Types of Genomic Variation: Large alterations

3. **Copy number changes**
   - Germline copy number variant (CNV) or polymorphism (CNP)
   - Somatic copy number variant (CNV) or alterations (CNA)
   - Size > 1 kbps, typically mega-bases (depending on resolution)
4. **Structural rearrangements**
   - Germline or Somatic structural variant (SV)
   - Simple events: deletion, duplication, inversion, translocation
   - Single nucleotide resolution for breakpoints
   - Size > 20 bps, typically kilo-bases to mega-bases



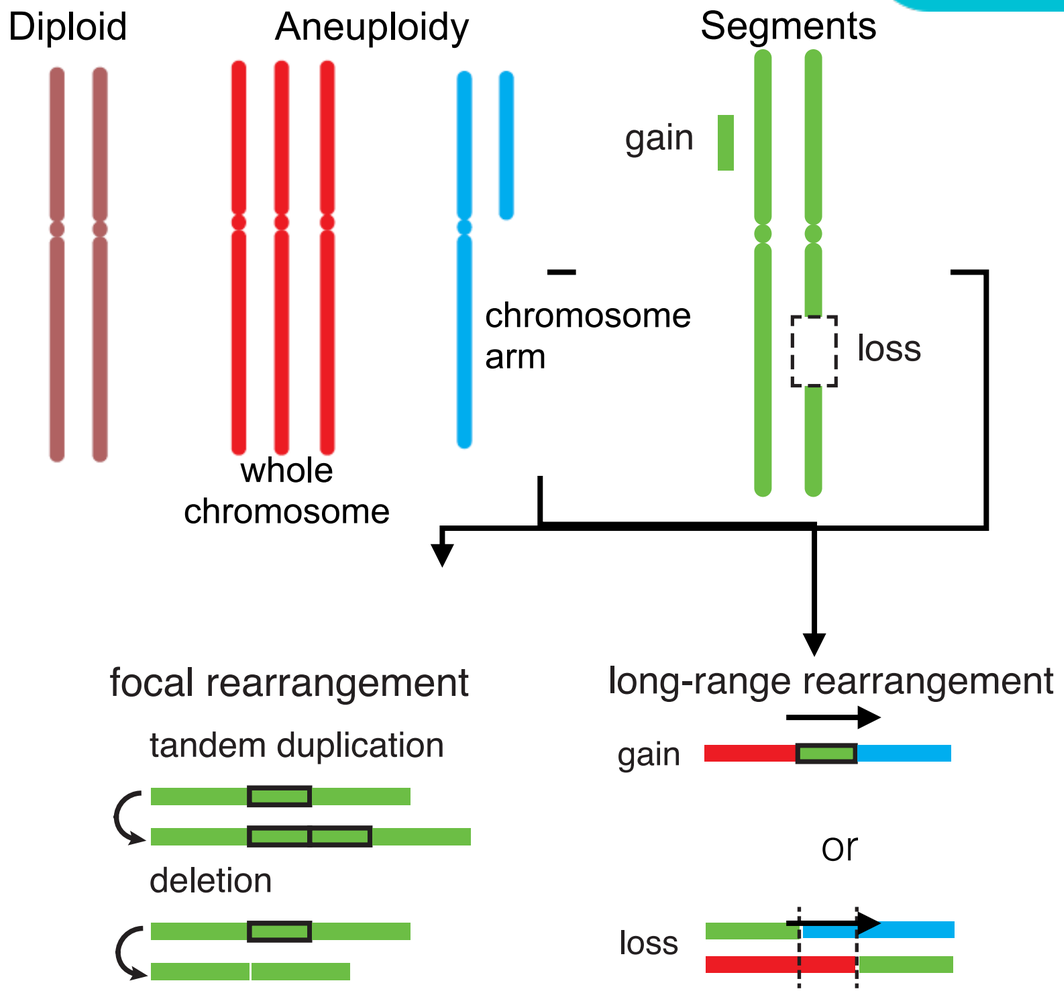
Types of Genomic Variation in Cancer

Single nucleotide variant



Insertion-Deletion (INDEL)

Copy number alterations



Structural rearrangements

Tumors exhibit different levels of heterogeneity

Across patient populations:

1. **Cancer types**: between primary tumors of different organs or tissue-of-origin (eg. Breast and lung cancers)

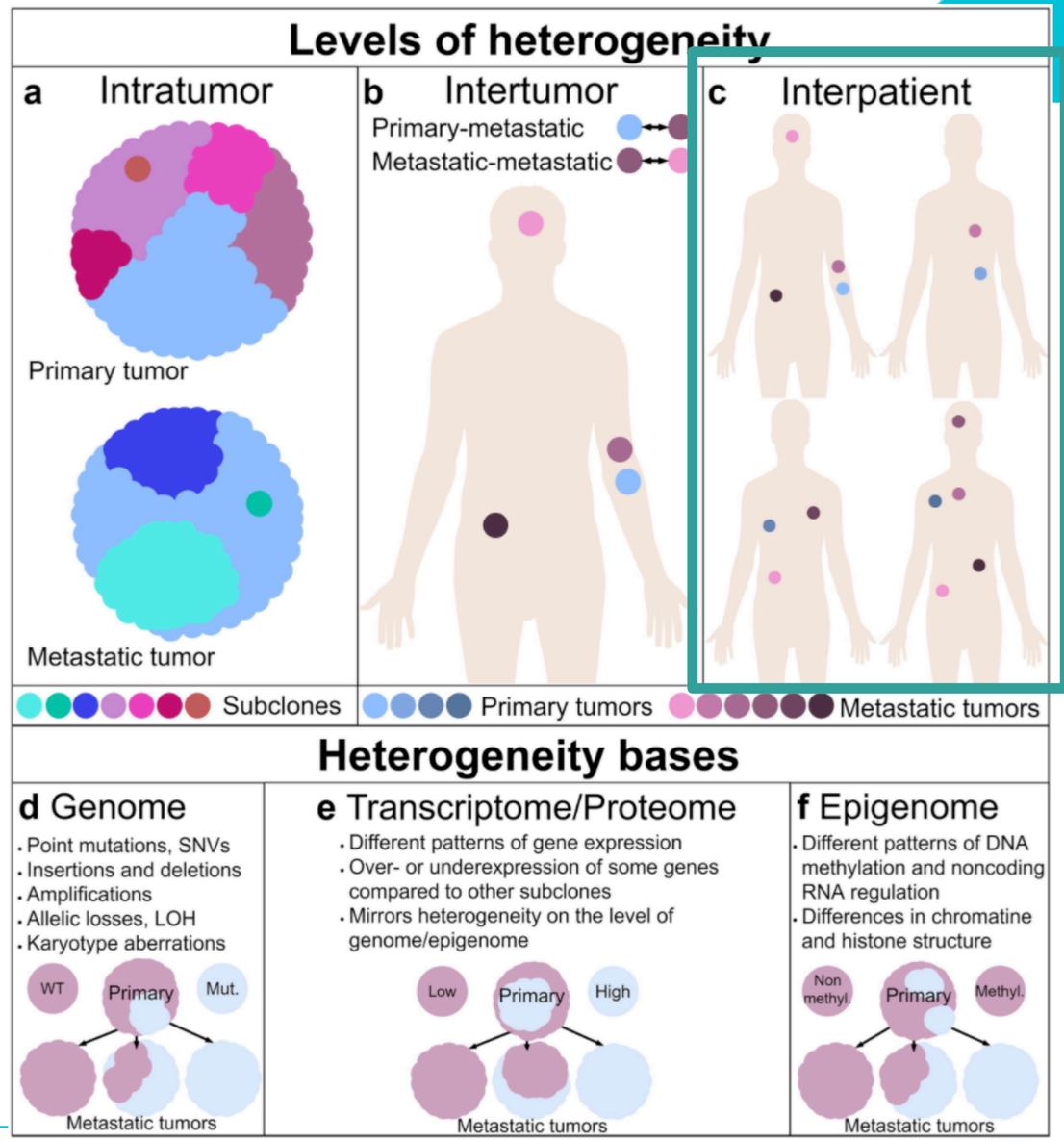
2. **Tumor subtypes**: between subset of patients with tumors having similar molecular features (e.g. ER+ and ER- breast cancers)

3. **Same-subtype**: between tumors from different patients

Within an individual patient:

4. **Inter-tumor**: between tumors within a patient

5. **Intra-tumor heterogeneity**: between cells within a tumor lesion (e.g. tumor clones, stromal cells, infiltrating lymphocytes)



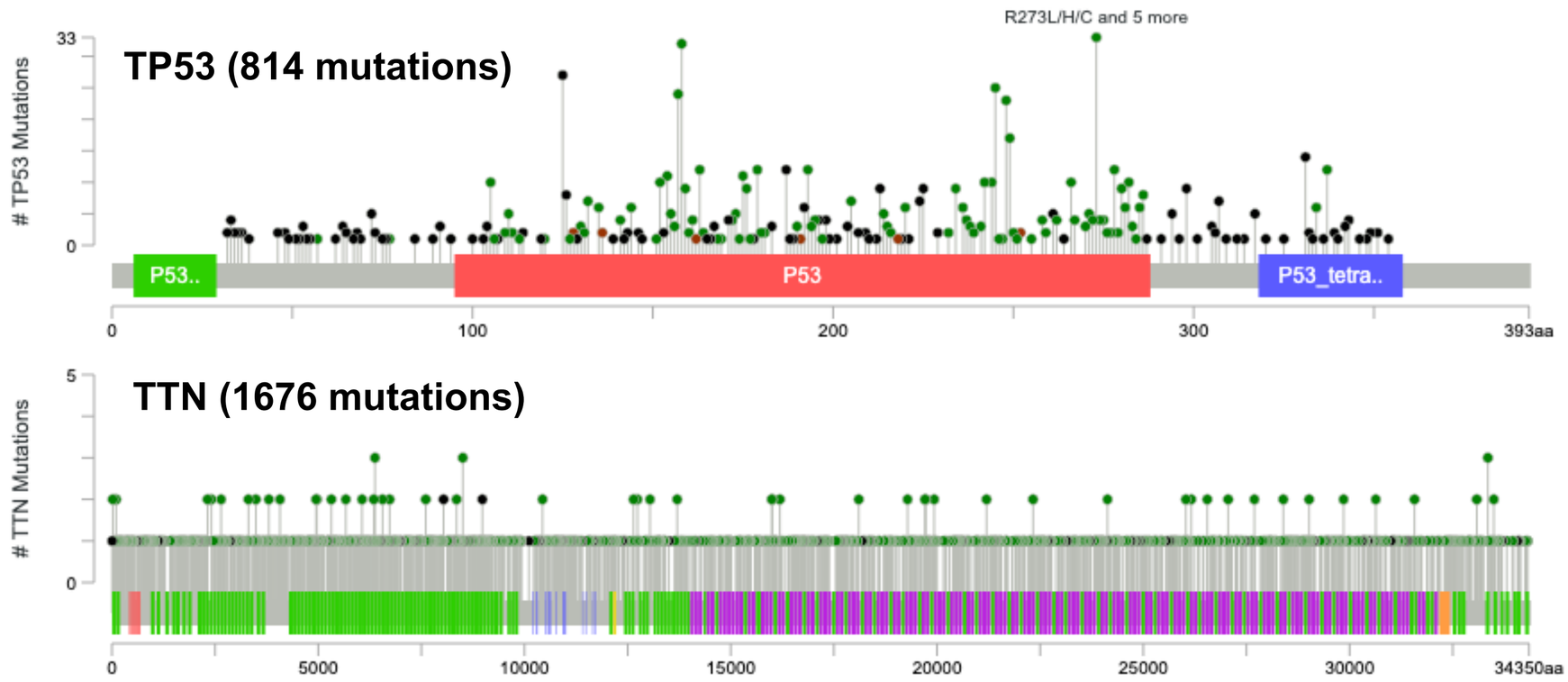
Grzywa et al. *Transl Oncol.* **10**:956-75 (2017)

Cancer Genes: Driver vs Passenger Genomic Alterations

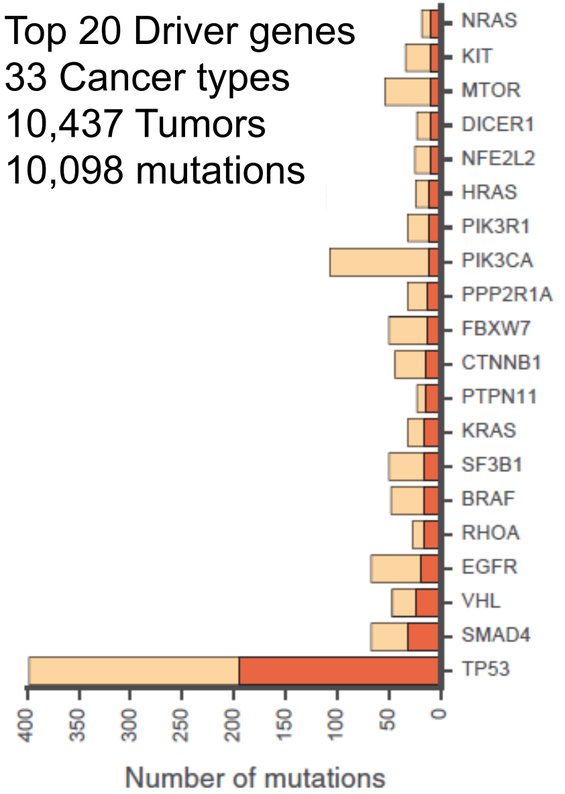
How do we find the mutated genes that ***drive*** cancer?

- **Significantly Mutated Genes:** recurrently mutated genes in patient cohorts
- Account for covariates (e.g. gene length, expression, replication timing)

1144 Lung Cancers



Top 20 Driver genes
33 Cancer types
10,437 Tumors
10,098 mutations



https://www.cbioportal.org/

Bailey et al. *Cell* **173**:371-85 (2018)

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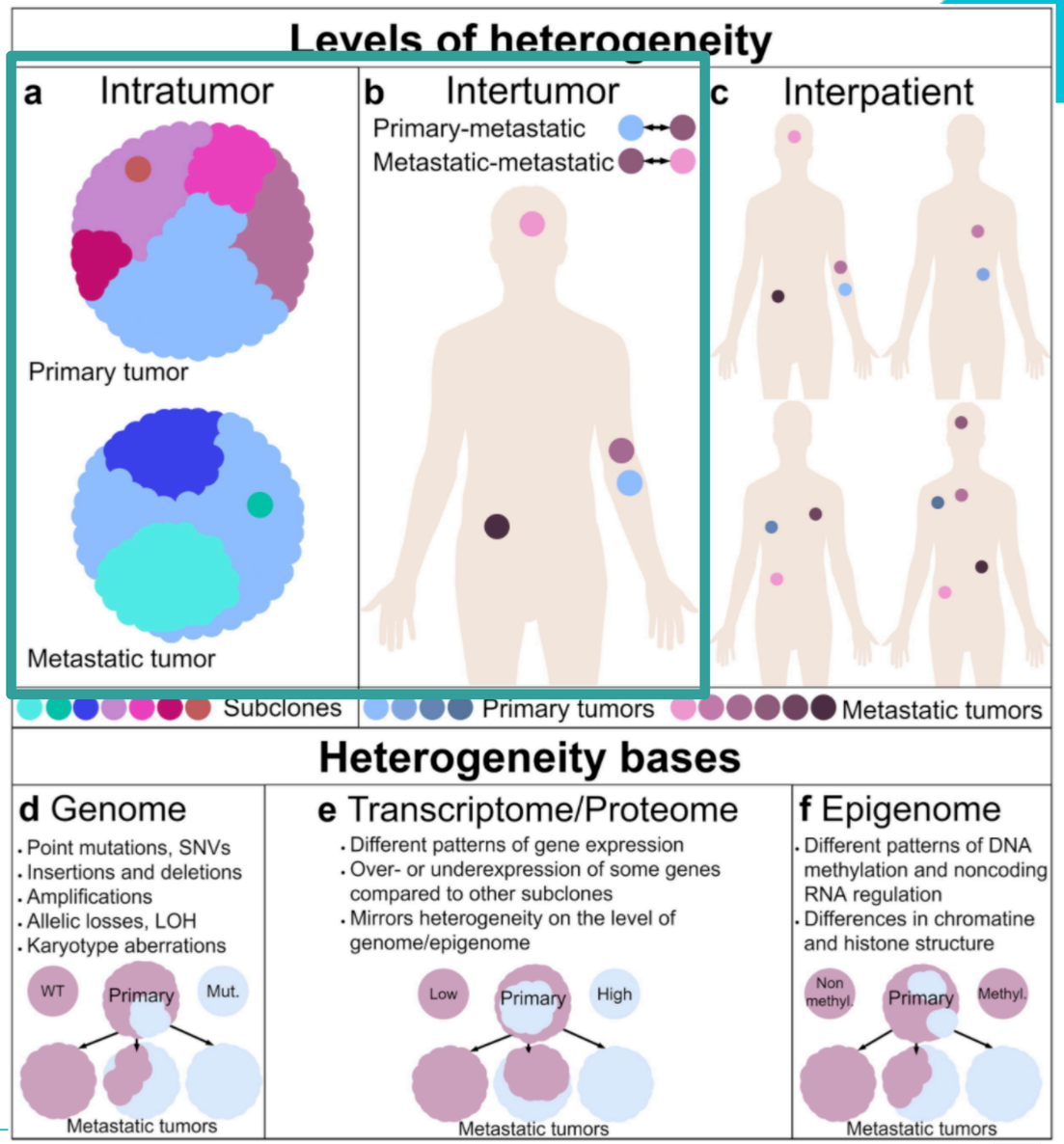
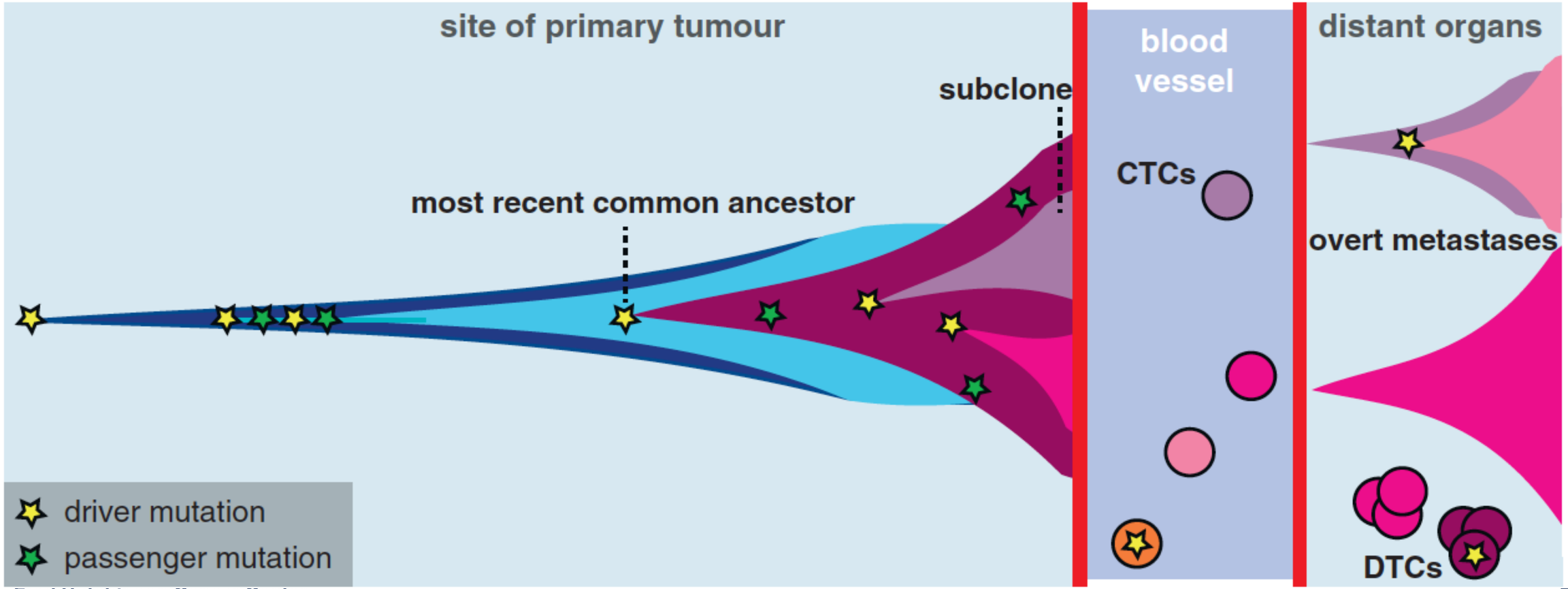


Grzywa et al. *Transl Oncol.* **10**:956-75 (2017)

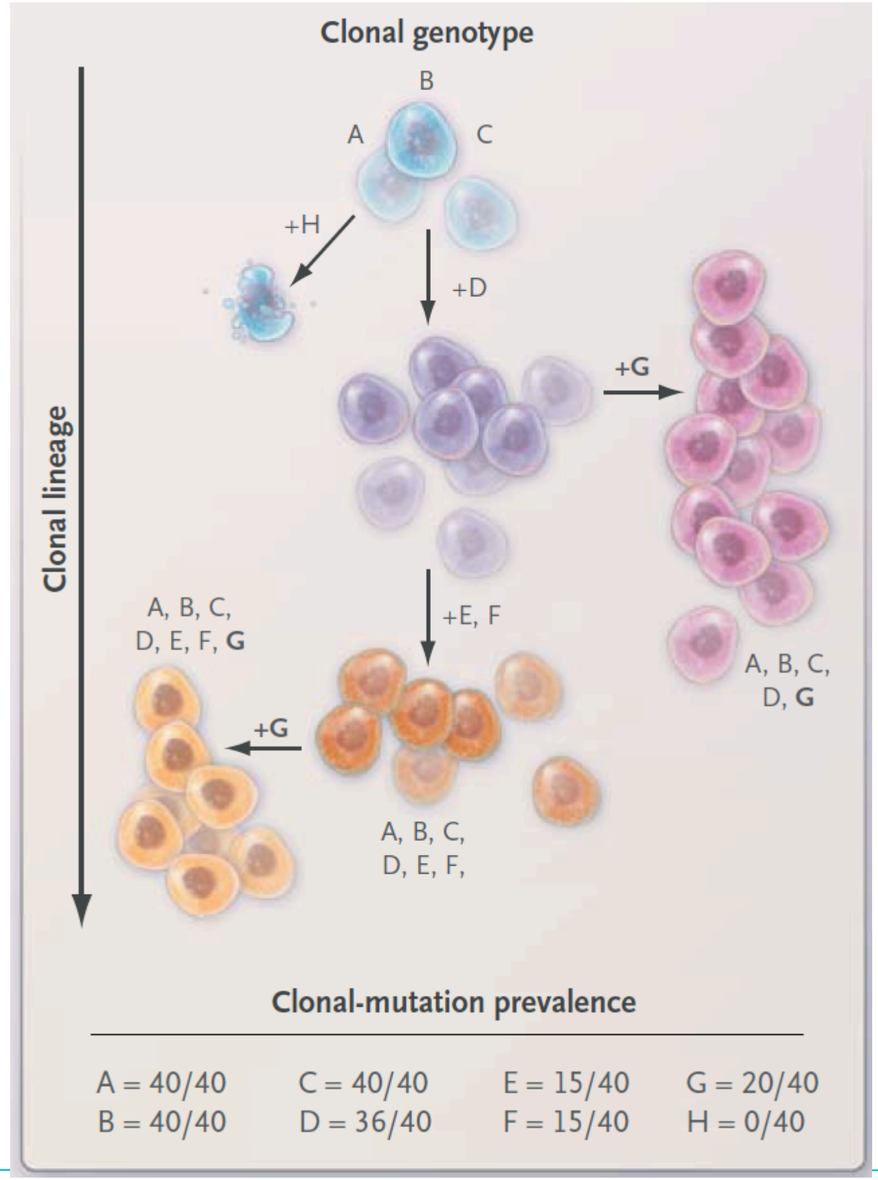
Tumors undergo genome evolution and clonal expansion

- Clonal diversity may have implications for treatment resistance
- Dynamics of clones can change in the blood and metastases



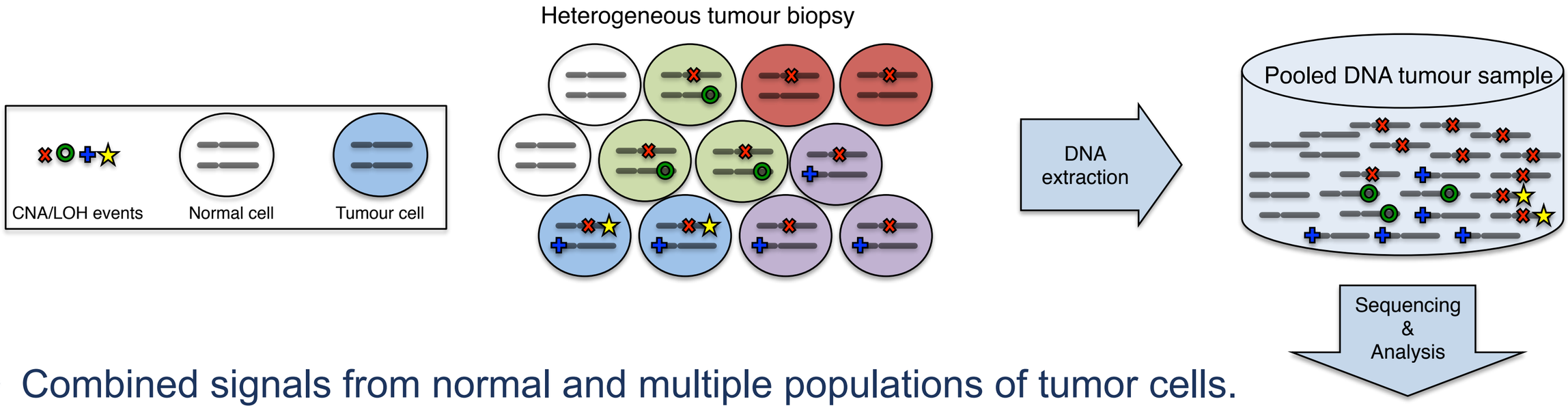
Van Loo and Voet. *Curr Opin Genet Dev* (2014)

Tumor genome evolution selects for cellular phenotypes



Aparicio & Caldas. *NEJM.* **368**:842-51 (2013)

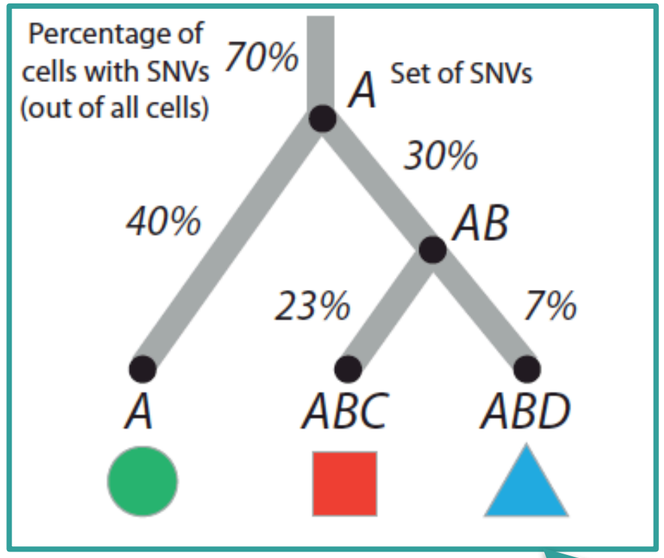
Inferring intra-tumor genomic heterogeneity from sequencing



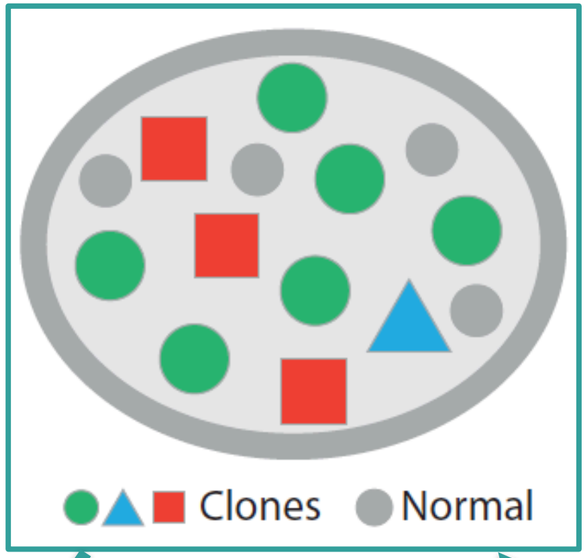
- Combined signals from normal and multiple populations of tumor cells.
- Cellular prevalence: proportion of tumor cells harboring event
- Discuss further in Lecture 4…

Inferring evolutionary history of a tumor from sequencing

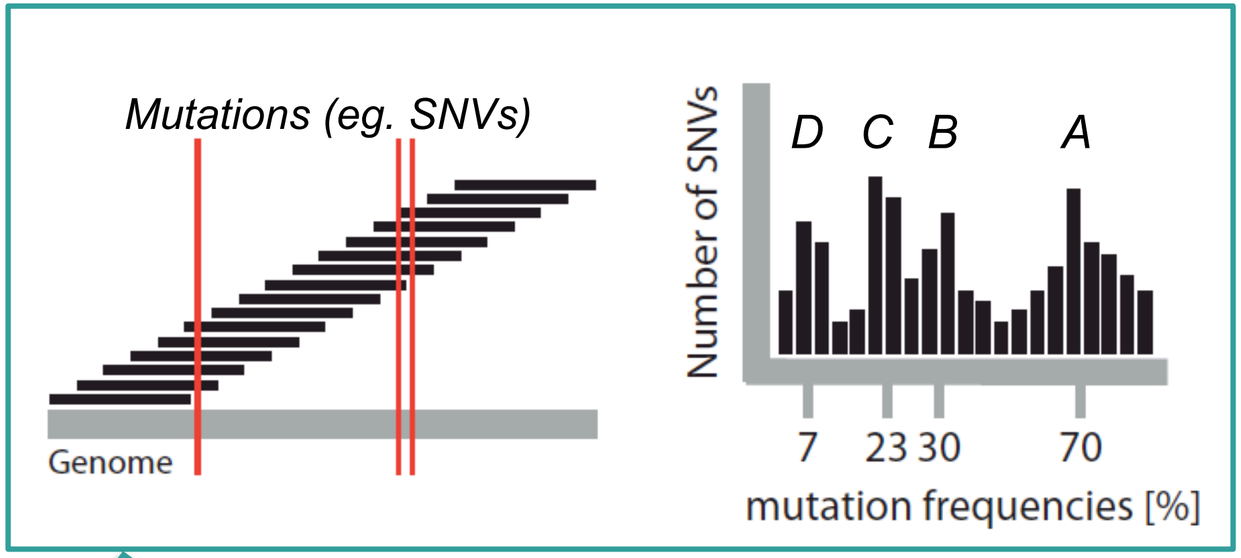
Evolutionary History



Clonal Cell Populations



Sequencing Data



3. Infer evolutionary (phylogenetic) tree

2. Infer clonal prevalence

1. Mutation Calling & Analysis

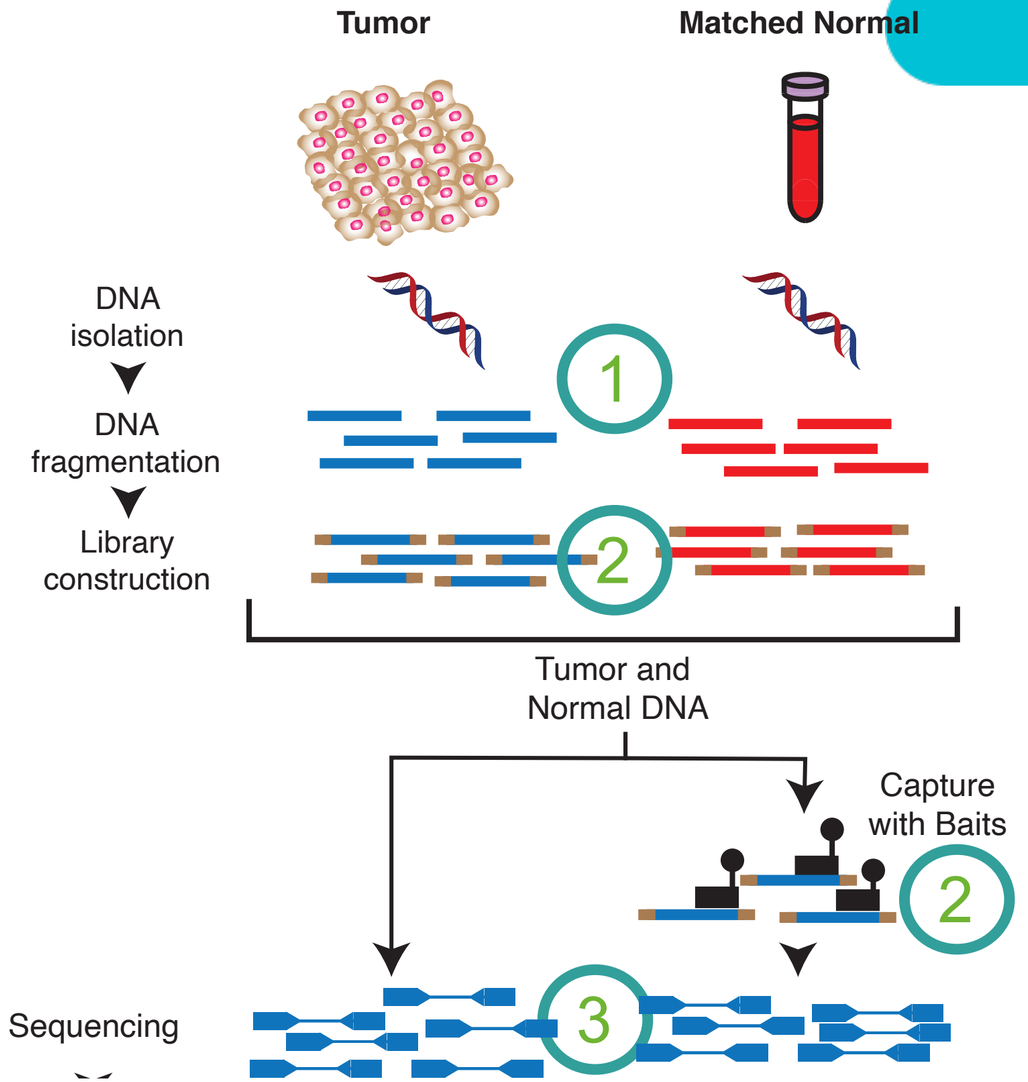
Adapted from Beerenwinkel et al. *Syst. Biol.* **64**:e1-25 (2015)

2. Overview of Cancer Genome Analysis

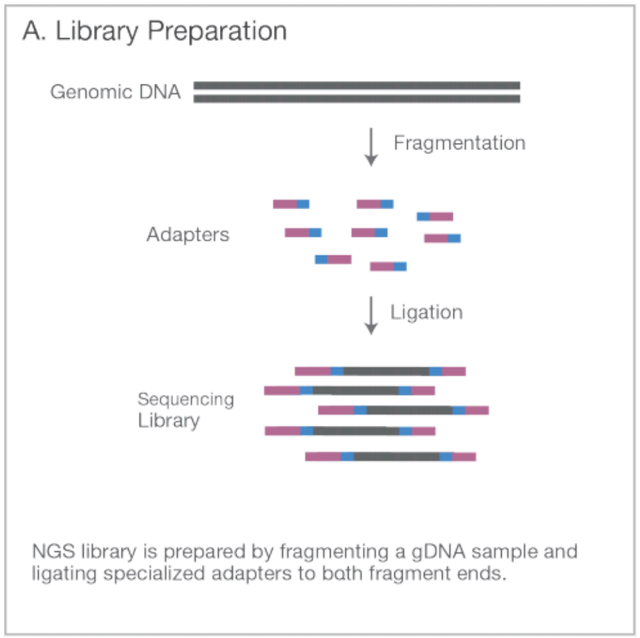
- Computational strategy and workflow
- Tumor DNA sequencing
- Whole genome vs whole exome vs targeted sequencing
- Types of genomic alterations predicted from tumor sequencing
- Methods/tools/algorithms in following lectures

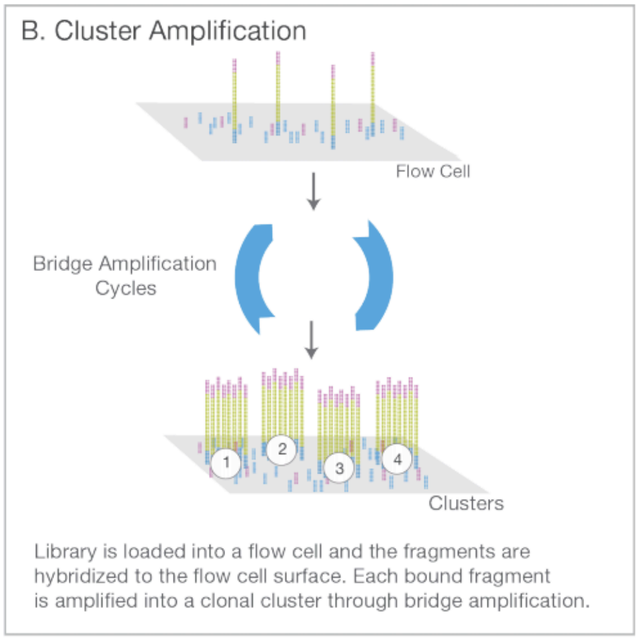
General Workflow of Tumor Genome Sequencing (1)

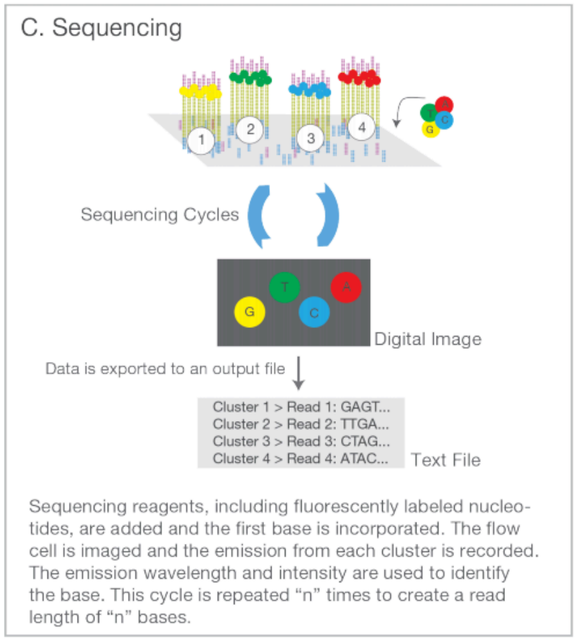
- Tumor and Normal pairing
  - Distinguish somatic and germline alterations
- Capture baits can be used to select regions
  - e.g. whole exome or targeted gene panels
- Potential sources of error can arise
  1. 8-oxoG transversions (C>A/G>T)
  2. PCR errors and GC content bias
  3. Sequencing errors

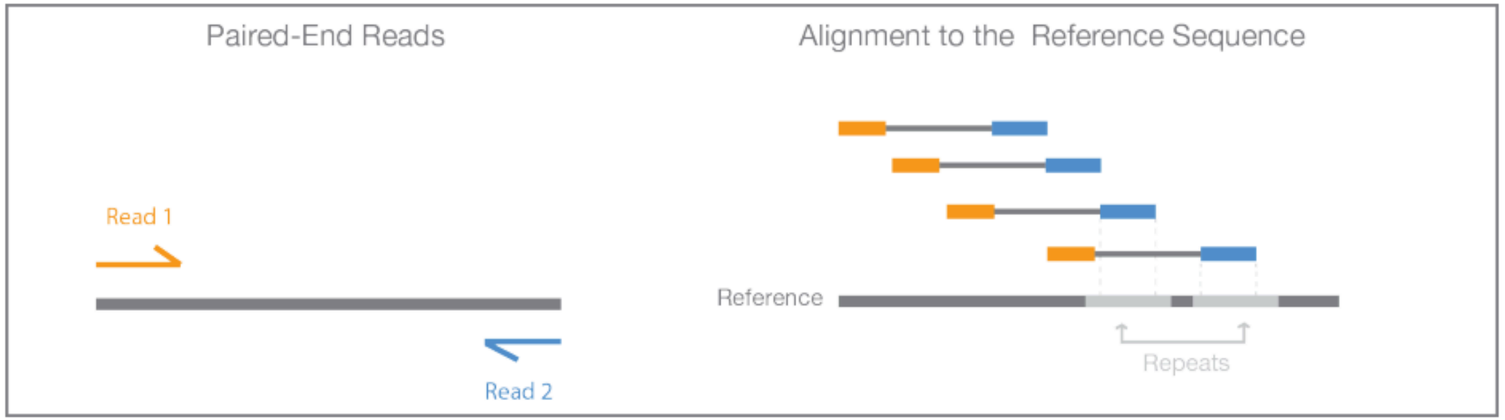


Genome Sequencing: Massively Parallel Sequencing



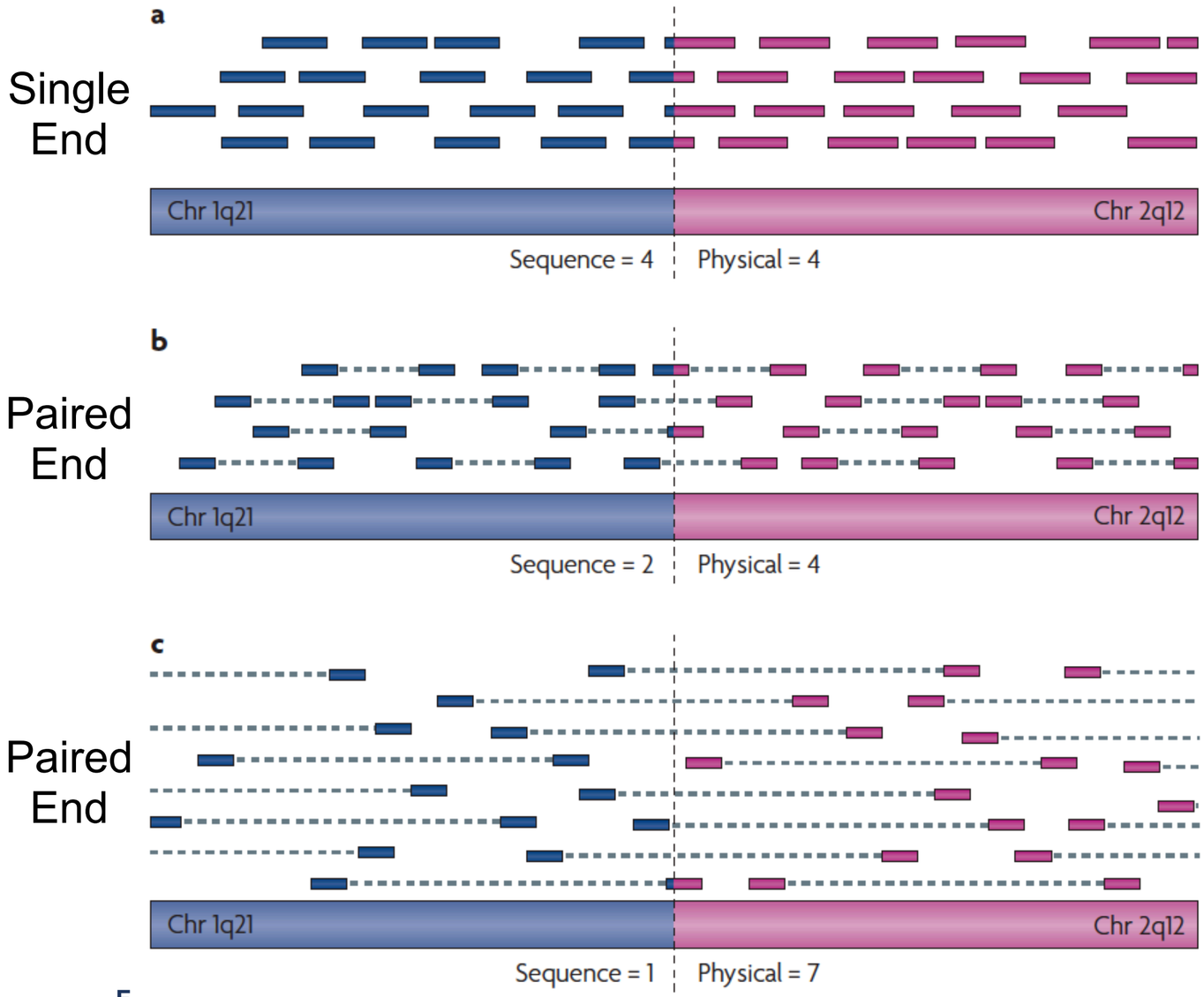






https://www.illumina.com/content/dam/illumina-marketing/documents/products/illumina_sequencing_introduction.pdf

Genome Sequencing: Sequence vs Physical Coverage



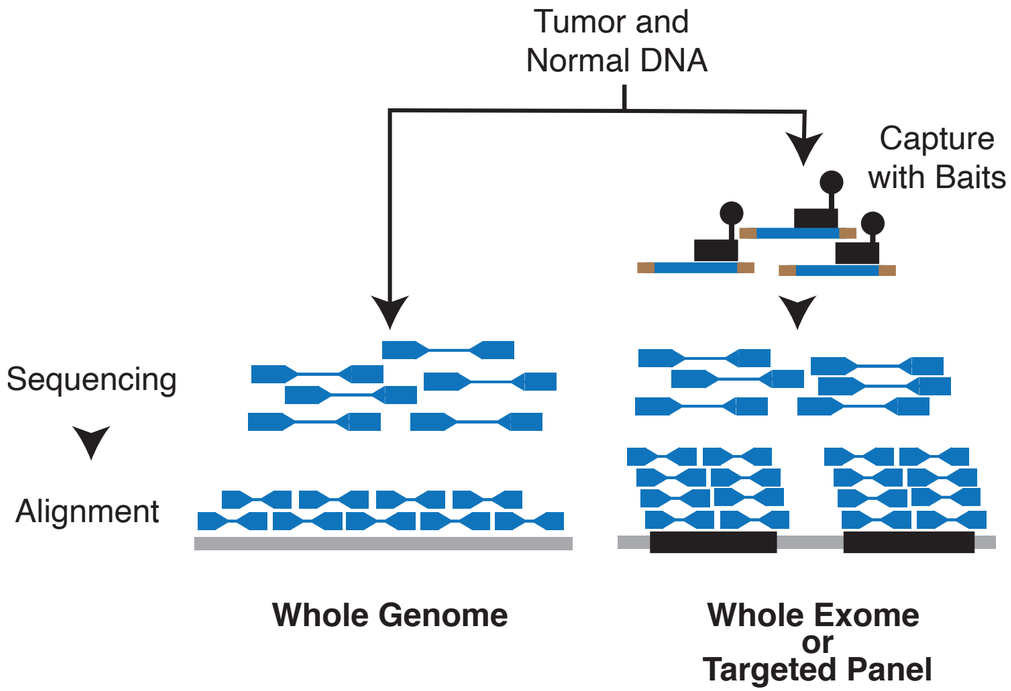
Sequence Coverage = number of sequenced reads spanning locus

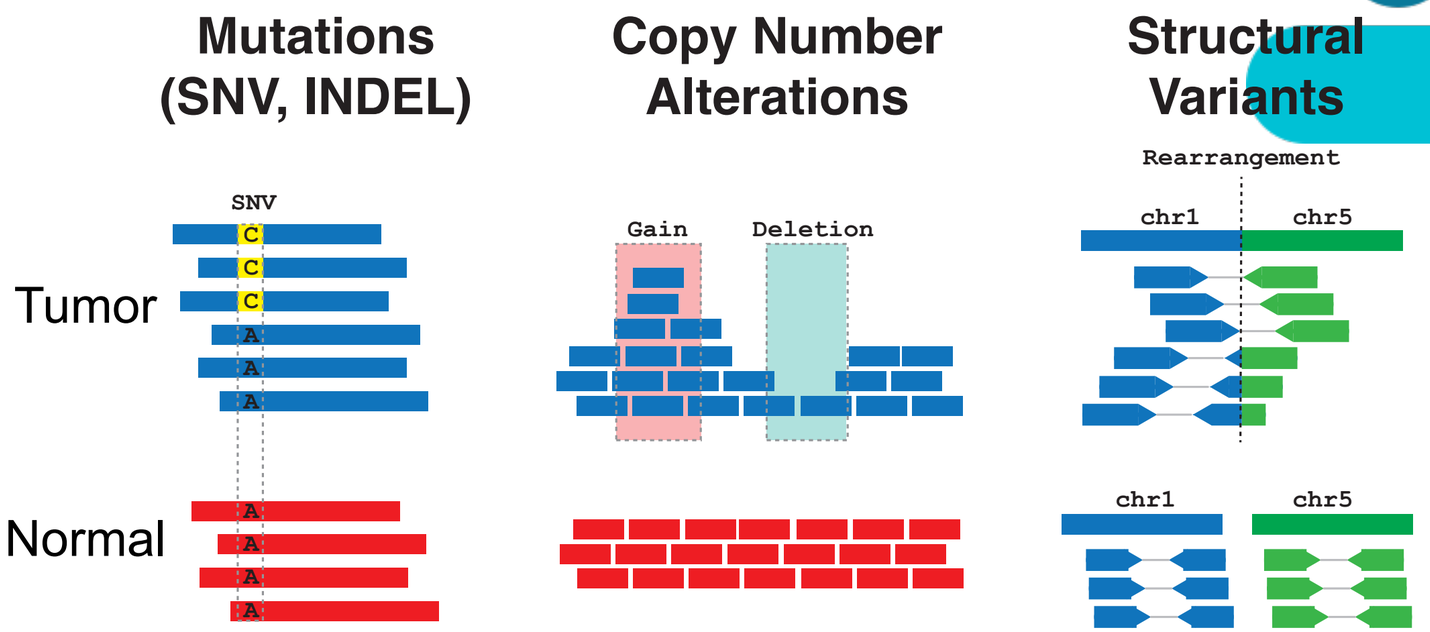
Physical Coverage = number of DNA fragments spanning locus

- Mutation detection rely on sequence coverage
- Rearrangement detection rely on both

Meyerson, Gabriel & Getz. *Nature Review Genetics* **11**:685-96 (2010)

General Workflow of Tumor Genome Sequencing (2)

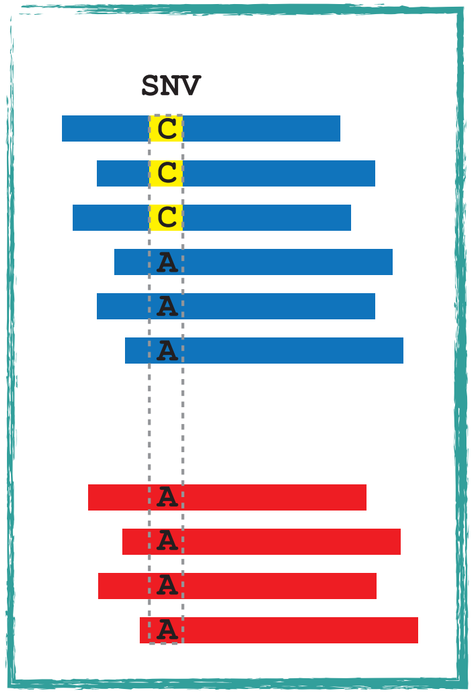




Whole Genome Sequencing	Whole Exome Sequencing	Targeted Gene Sequencing
• Genome-wide (unbiased) • 0.1-100x genome coverage	• Exons (2% of genome) • 50-500x target coverage	• Target regions (1-5Mb) • 100-25000x target coverage
• More sequencing required • Expensive	• Less sequencing required • Cost-effective	• Least sequencing required • Panel design costs
• Coding/Non-coding mutations • Copy number alterations • Structural variation	• Coding mutations (all genes) • Copy number alterations • Gene fusions rearrangements	• Coding mutations (selected) • Targeted rearrangements

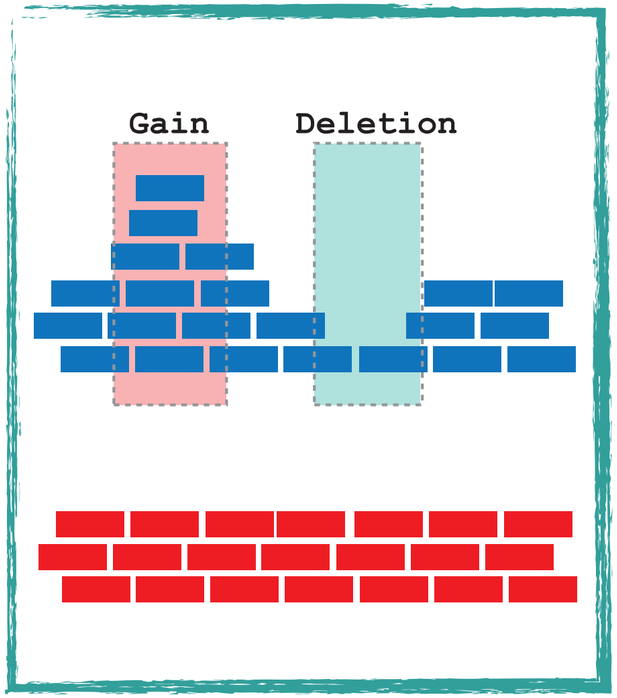
Types of Genomic Alterations Predicted from Sequencing

Mutations (SNV, INDEL)



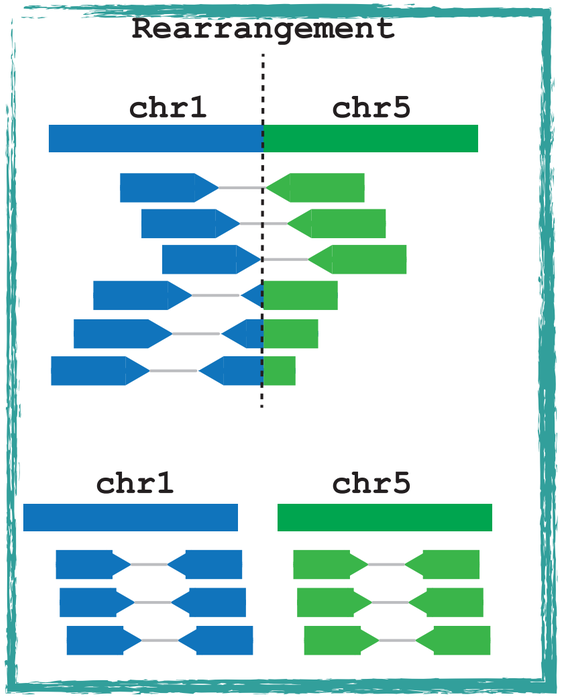
Lecture 2

Copy Number Alterations



Lecture 3

Structural Variants



Lecture 4

Genome Sequencing: International Consortia & Projects

1000 Genomes Project (https://www.internationalgenome.org/)

UK10K (https://www.uk10k.org/)

The 100,000 Genomes Project (https://www.genomicsengland.co.uk/)

- Rare disease, cancer, infectious disease

Genome 10K Project (https://genome10k.soe.ucsc.edu/)

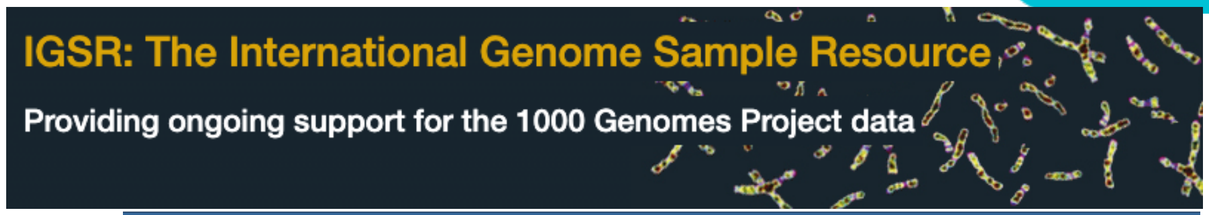
- Genomic “zoo” of 16,000 vertebrate species

Exome Aggregation Consortium (ExAC) (http://exac.broadinstitute.org/)

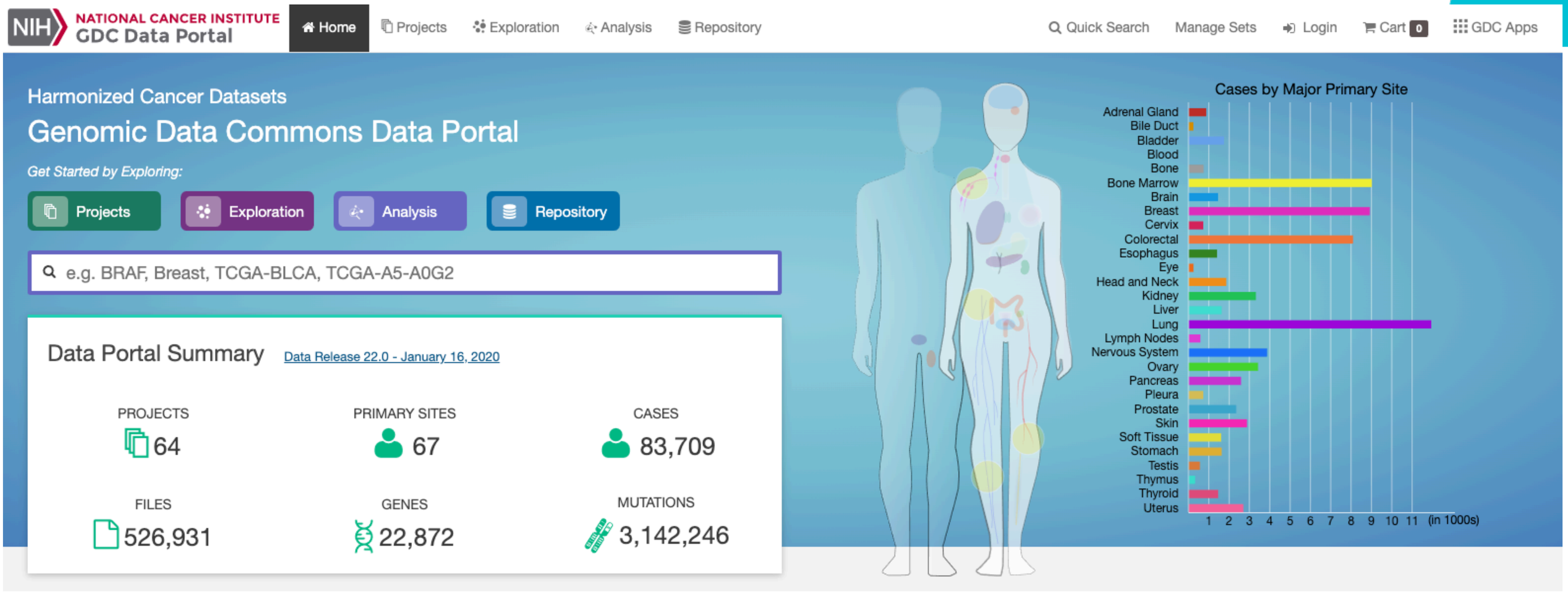
Genome Aggregation Database (gnomAD) (https://gnomad.broadinstitute.org/)

The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/)

International Cancer Genome Consortium (ICGC) (https://icgc.org/)



Cancer Genome Sequence Data: Databases & Online Resources



https://portal.gdc.cancer.gov/

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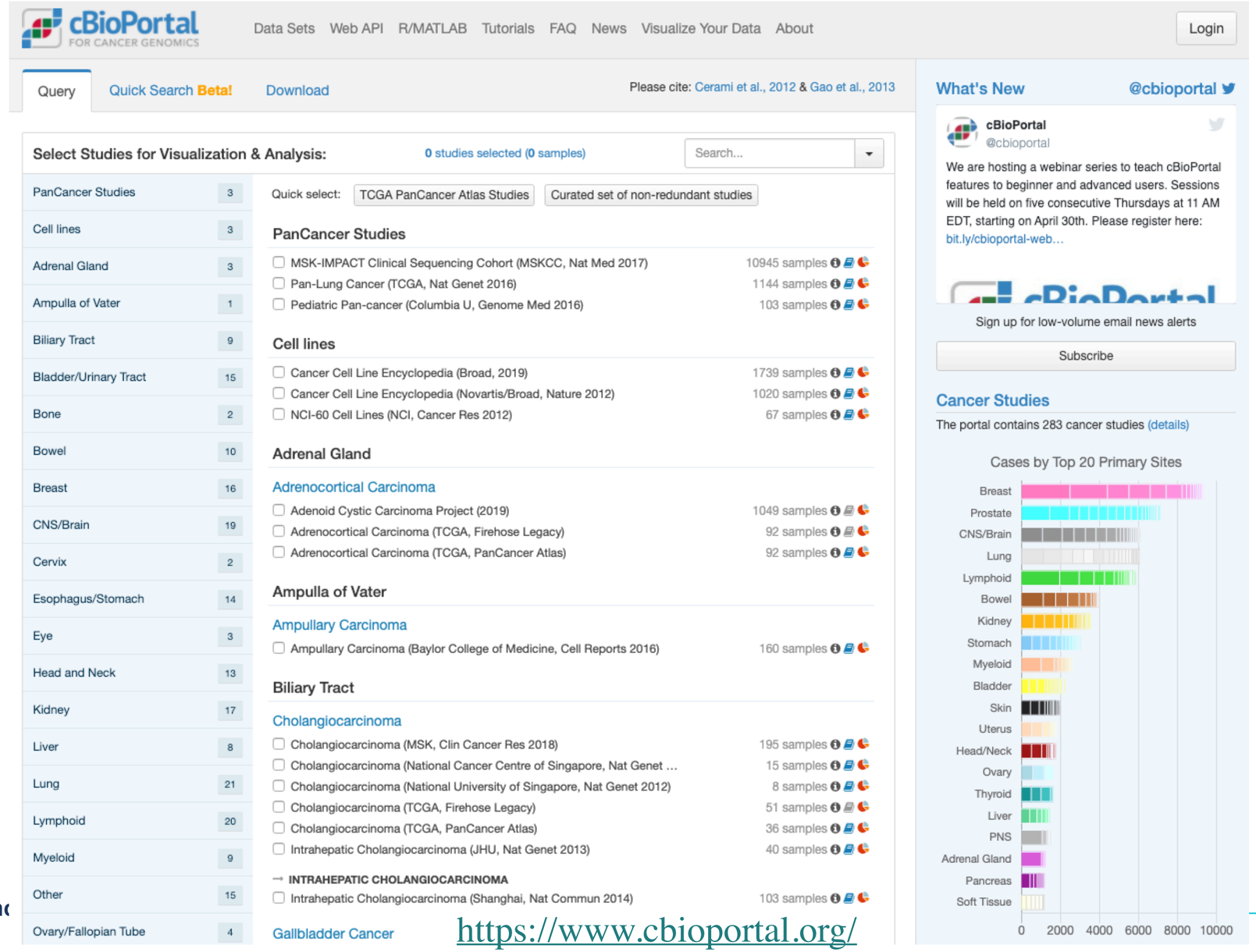

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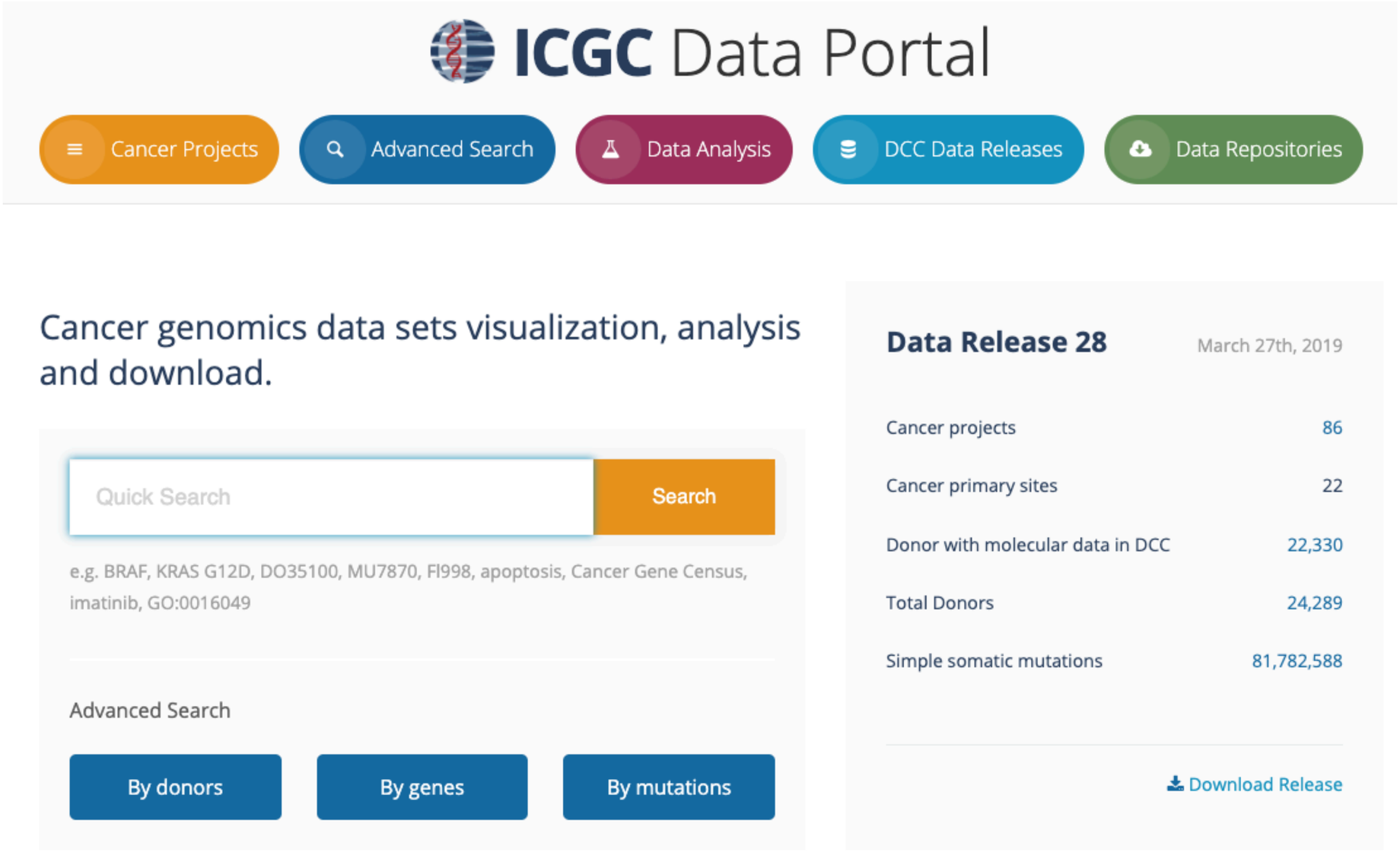


3. Primer on statistical modeling

- Probability
  - Unsupervised learning, probability rules & Bayes' theorem
  - Binomial distribution, Bayesian statistics
  - Beta-binomial model example
- Mixture models, EM inference
- References:
  - Murphy, K. (2012). Machine Learning: A Probabilistic Perspective. MIT Press. ISBN: 9780262018029
  - Bishop, C. M. (2006). Pattern Recognition and Machine Learning (Information Science and Statistics). Springer. ISBN: 0387310738
  - https://www.cs.ubc.ca/~murphyk/Teaching/CS340-Fall06/reading/bernoulli.pdf

Sequencing Data Analysis Requires Probabilistic Models

- Sequencing data contain uncertainty due to
  - Technical noise from imperfect measurements & errors
  - Biological features in the signal measurements
- How do we predict genomic alterations accounting for these features and noise?
  - Need approaches to learn the patterns of these features from the data…

Types of machine learning:

- Supervised: output data y, input data $\boldsymbol{x}$, and *training set* $D = \{(\boldsymbol{x}, y)\}$
  - Classification (y are labels), Regression (y is continuous)
- Unsupervised: Only given input data $D = \{\boldsymbol{x}\}$, *learn the patterns of the data*
  - E.g. clustering input data $\boldsymbol{x}$ into K clusters by estimating their assignments $\boldsymbol{z}$

Primer: Probability Theory

Let X be a random variable. The probability for the event $X = x$ for some value x is $p(X = x)$ or $p(x)$ for short. Let Y be another random variable.

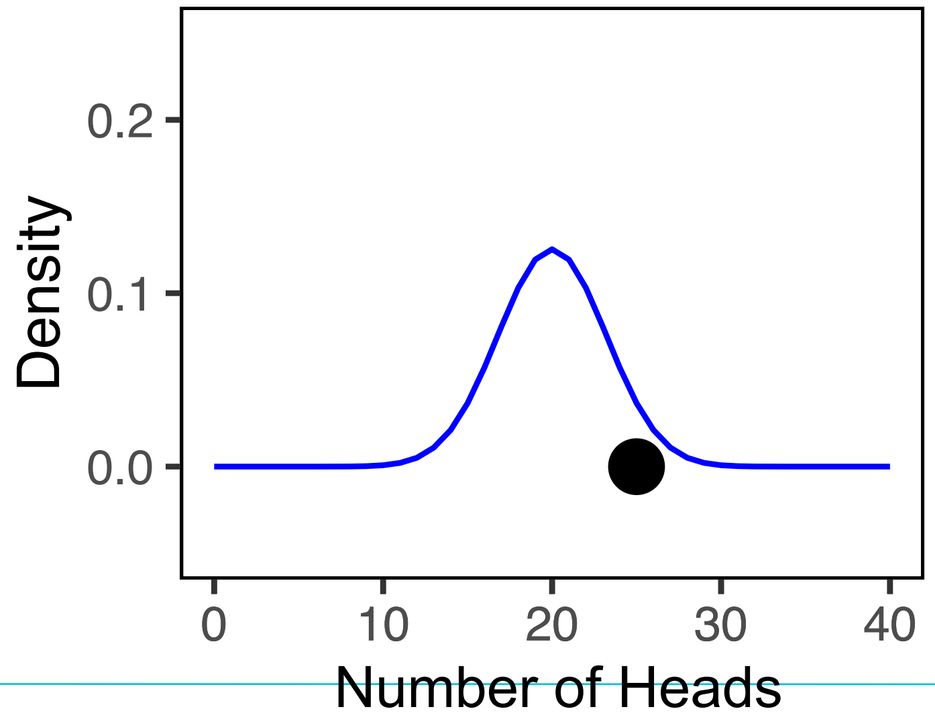
Probability Rules

- **Sum rule:** $p(X) = \sum_Y p(X, Y)$
- **Product rule:** $p(X, Y) = p(Y \mid X)p(X)$ and $p(Y, X) = p(X \mid Y)p(Y)$
- Conditional Probabilities: $p(Y \mid X) = \frac{p(X, Y)}{p(X)}$
- Marginal Probabilities: $p(X) = \sum_Y p(Y, X) = \sum_Y p(X \mid Y)p(Y)$
- **Bayes' Theorem (rule):** $p(Y \mid X) = \frac{p(X, Y)}{p(X)} =$

Probability distribution: Binomial

Binomial Distribution: Referee Coin Toss Example

- A referee has a coin that he uses to decide which team gets first possession. She tossed the coin N times last season, once per game. We assume this coin was fair and had a probability $\mu = 0.5$ for showing a heads. We kept track of the number of heads x that appeared.
- What is the probability of seeing a specific number of heads? e.g. $x = 25$ out of $N = 40$ tosses



Probability distribution: Binomial

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Probability mass function

- Let X be the random variable representing the number of heads. If the probability of heads is μ, then X has a binomial distribution, $X \sim Bin(N, \mu)$ or $p(X = x \mid N, \mu) = Bin(x \mid N, \mu)$ where

$$Bin(x \mid N, \mu) = \binom{N}{x} \mu^x (1 - \mu)^{N-x}$$

$\binom{N}{x}$ number of ways the 25 heads is observed among the sequence of 40 tosses.

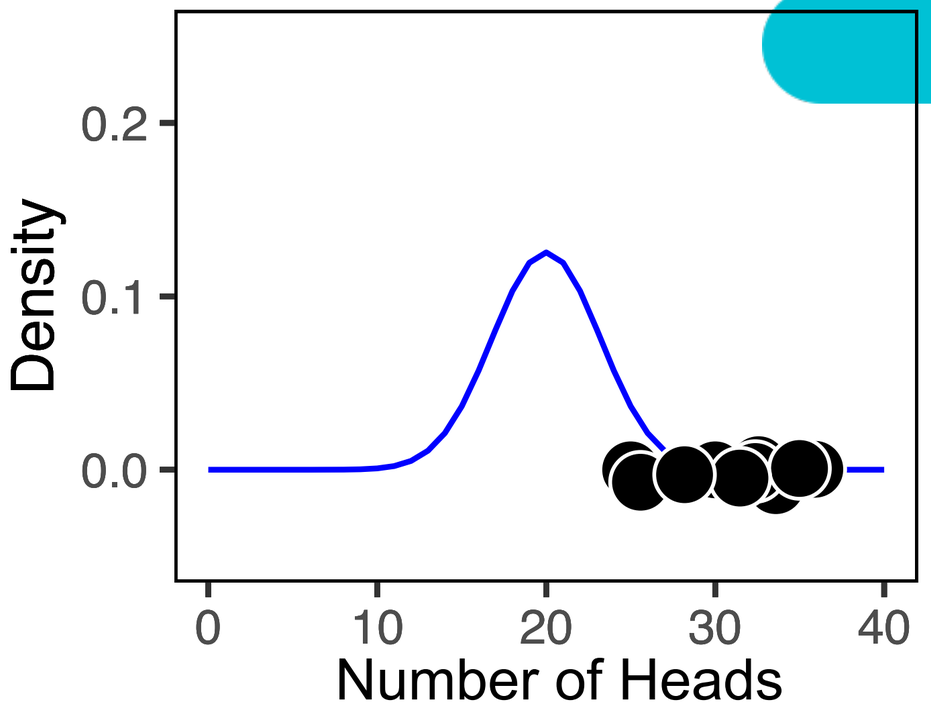
- Our coin-toss example: for $x = 25$ out of $N = 40$ and a fair coin $\mu = 0.5$

Binomial likelihood model

- Suppose there are T different referees who toss the *same* coin $\boldsymbol{N} = \{1,\ldots,N_T\}$ times and come up with head counts $\boldsymbol{x} = \{1,\ldots,x_T\}$.
- Assuming the referees' tosses are *independent* and *identically distributed* (**iid**), what is the probability of observing the head counts from *all referees* given the coin (e.g. $\mu = 0.5$)?

$$p(x_{1:T} \mid N_{1:T}, \mu) = \prod_{i=1}^{T} Bin(x_i \mid N_i, \mu)$$ **Likelihood**

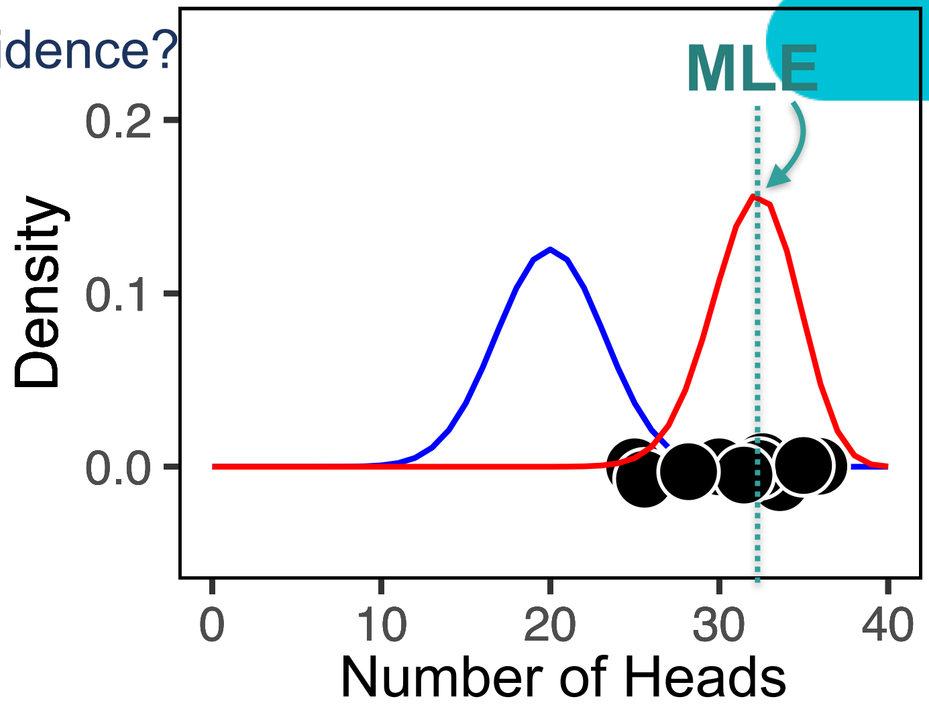
- What if the coin wasn't fair and the probability of heads, μ, might not be 0.5?



	# of tosses (*N*)	# of heads (*x*)
Referee 1	40	25
Referee 2	42	35
Referee 3	39	27
Referee T	x_T	N_T

Maximum likelihood estimation (MLE)

- What is the probability of heads, μ, of this coin given the evidence?
- We can estimate this model *parameter* using ***maximum likelihood estimation***



$$p(x_{1:T} | N_{1:T}, \mu) = \prod_{i=1}^{T} Bin(x_i | N_i, \mu)$$

Likelihood

$$\log p(x_{1:T} | N_{1:T}, \mu) = \sum_{i=1}^{T} \log Bin(x_i | N_i, \mu)$$

Log-likelihood

$$\hat{\mu} = \frac{\sum_{i=1}^{T} x_i}{\sum_{i=1}^{T} N_i}$$

MLE

https://www.cs.ubc.ca/~murphyk/Teaching/CS340-Fall06/reading/bernoulli.pdf

Bayesian Statistics: Prior distribution for model parameters

Likelihood for Binomial Model

$$p(x_{1:T} \mid N_{1:T}, \mu) = \prod_{i=1}^{T} Bin(x_i \mid N_i, \mu)$$ **Likelihood**

	# of tosses (N)	# of heads (x)	Prop. of heads
Referee 1	40	25	0.63
Referee 2	42	35	0.83
Referee 3	39	27	0.69
Referee T	x_T	N_T	x_T/N_T

- MLE uses the evidence to estimate parameter $\hat{\mu}$ but our sample size is small and MLE may **overfit**
- **Zero count or sparse data problem:** If you have a bad record keeper who only tallies coin tosses from referees who never tosses a tail, then does that mean the concept of tails on a coin does not exist at all?
- Can we capture a more natural expectation of how a coin might behave? Also, what if we have some knowledge that the coin might be biased?

Prior Distribution for binomial parameter, μ

- The proportion of heads is between 0 and 1 ($\mu \in [0,1]$) and can be sampled from a distribution itself
- μ can be drawn from a Beta distribution, which is in the interval $[0,1]$, with ***hyper-parameters*** α and β

$$\mu \sim Beta(\alpha, \beta)$$
$$p(\mu) = Beta(\mu \mid \alpha, \beta)$$ **Prior**

Bayesian statistics: Posterior for Beta-Binomial Model (1)

Binomial likelihood and Beta prior

- T different head counts $\boldsymbol{x} = \{1,\ldots,x_T\}$ for $\boldsymbol{N} = \{1,\ldots,N_T\}$ sets of tosses and a ***prior*** distribution on μ (prob. of heads)

$$p(x_{1:T} \mid N_{1:T}, \mu) = \prod_{i=1}^{T} Bin(x_i \mid N_i, \mu) \qquad \textbf{Likelihood}$$

$$p(\mu) = Beta(\mu \mid \alpha, \beta) \qquad \textbf{Prior}$$

- To estimate parameter μ in a Bayesian framework
  - We need the ***posterior***, $p(\mu \mid \boldsymbol{x})$, but only have $p(\boldsymbol{x} \mid \mu)$ and $p(\mu)$
- Recall Bayes' Theorem:

$$\underbrace{p(Y \mid X)}_{\textbf{Posterior}} = \frac{\overbrace{p(X \mid Y)}^{\textbf{Likelihood}}\overbrace{p(Y)}^{\textbf{Prior}}}{\sum_{Y'} p(X \mid Y')p(Y')} \propto$$

- The ***posterior*** is our **belief state** by combining evidence from observations and our prior beliefs.

Bayesian statistics: Posterior for Beta-Binomial Model (2)

Beta-Binomial Model: Posterior distribution

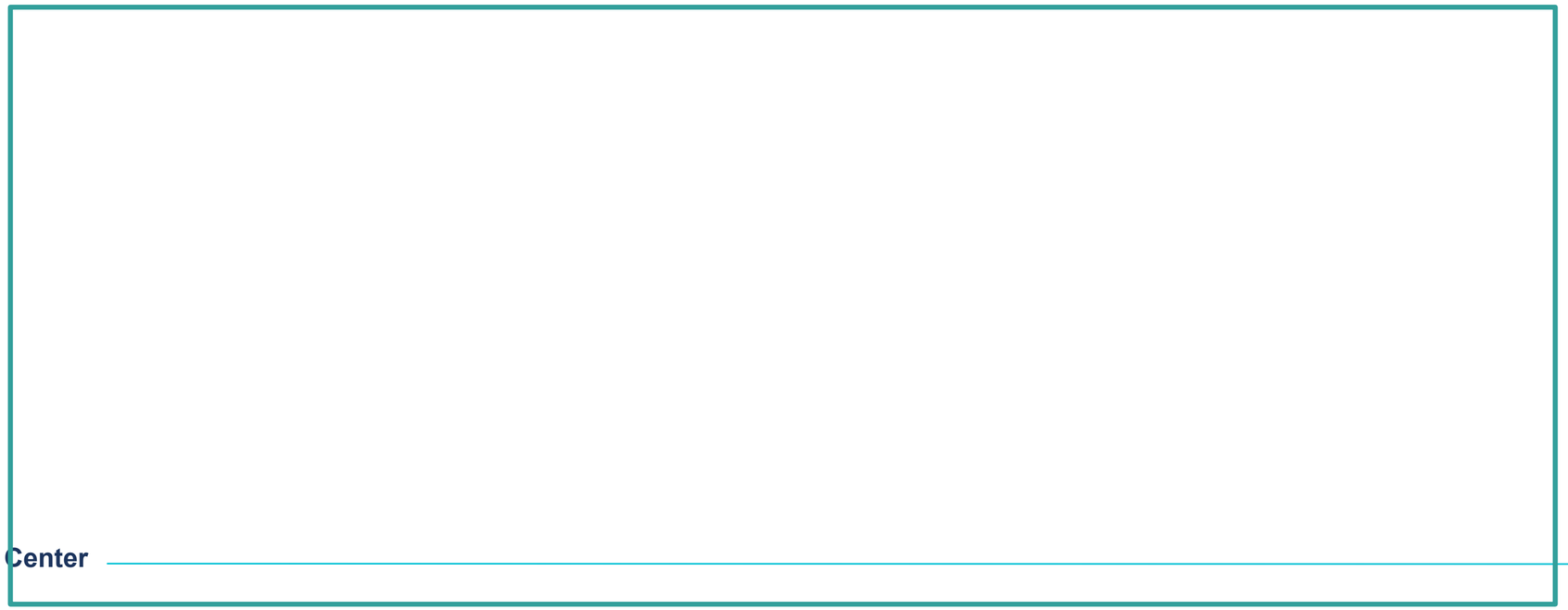
- To estimate the model parameter μ in a Bayesian framework, we compute the ***posterior***, $p(\mu \mid \boldsymbol{x})$

$$p(\mu \mid x_i) \propto Bin(x_i \mid N_i, \mu) \times Beta(\mu \mid \alpha, \beta)$$

- Beta is a ***conjugate prior*** for the binomial — *the product of binomial and Beta has the form of a Beta*

$$p(\mu \mid x_i) \propto Bin(x_i \mid N_i, \mu) \times Beta(\mu \mid \alpha, \beta) = Beta(\mu \mid x_i + \alpha, N_i - x_i + \beta)$$

Likelihood **Prior** **Posterior**



Bayesian statistics: Posterior for Beta-Binomial Model (2)

Beta-Binomial Model: Posterior distribution

- To estimate the model parameter μ in a Bayesian framework, we compute the ***posterior***, $p(\mu \mid \boldsymbol{x})$

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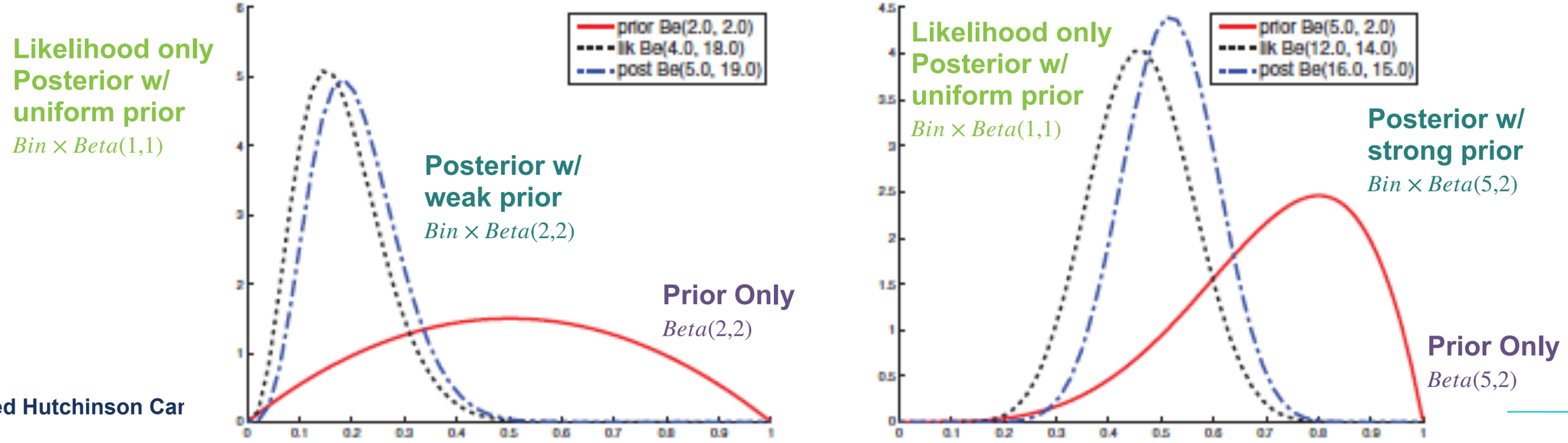
Likelihood **Prior** **Posterior**

Figure 3.6 in Murphy, K. (2012). Machine Learning: A Probabilistic Perspective. MIT Press



Bayesian statistics: MAP estimate

Beta-Binomial Model: Posterior distribution

$$p(\mu \mid x_i) \propto Bin(x_i \mid N_i, \mu) \times Beta(\mu \mid \alpha, \beta) = Beta(\mu \mid \underbrace{x_i + \alpha}_{\alpha'}, \underbrace{N_i - x_i + \beta}_{\beta'})$$

Posterior

- Then, what is the probability of heads, μ, of this coin given the **evidence** and the **prior**?

Maximum a posteriori (MAP) estimate

- From the posterior, we can estimate the parameter using the ***maximum a posteriori (MAP)***, $\hat{\mu}_{MAP}$
- MAP refers to the mode of the posterior distribution and the mode of a Beta is $\frac{\alpha - 1}{\alpha + \beta - 2}$
- Since the posterior has the form of a Beta distribution, then the MAP is $\frac{\alpha' - 1}{\alpha' + \beta' - 2}$

$$\alpha' = x_i + \alpha$$

$$\beta' = \left(N_i - x_i\right) + \beta$$

$$\hat{\mu}_{MAP} = \frac{x_i + \alpha - 1}{N_i + \alpha + \beta - 2}$$

MAP

Section 3.3 in Murphy (2012). Machine Learning: A Probabilistic Perspective. MIT Press

Mapping the Referee Example to Mutation Calling

Referee Coin Toss Example

Data

Referees $1,\ldots,T$

For each Referee i

- Coin Tosses: N_i
- Count of heads: x_i
- Count of tails: $N_i - x_i$

Parameters

Probability to draw coins: π_{fair}, π_{heads}, π_{tails}

Probability of heads for 3 types of coins

μ_{fair}, μ_{heads}, μ_{tails}

Responsibilities

Probability that Referee i used coin k: $\gamma(Z_i = k)$

Mutation Calling from Sequencing Data

Data

Genomic loci $1,\ldots,T$

For each locus i

- Depth (total reads): N_i
- Count of reference reads: x_i
- Count of variant reads: $N_i - x_i$

Parameters

Probability of genotypes: π_{AA}, π_{AB}, π_{BB}

Probability of reference base for 3 genotypes:

μ_{AA}, μ_{AB}, μ_{BB}

Responsibilities

Probability that locus i has genotype k: $\gamma(Z_i = k)$

Mixture Models: Online Tutorial and Resource

fiveMinuteStats (https://stephens999.github.io/fiveMinuteStats/)

by **Dr. Matthew Stephens**, Professor in Statistics & Human Genetics at University of Chicago

1. Introduction to mixture models with probabilistic derivations and R code
   - Examples with Bernoulli and Gaussian models
   - https://stephens999.github.io/fiveMinuteStats/intro_to_mixture_models.html
2. Introduction to EM with Gaussian Mixture Model example and R code
   - https://stephens999.github.io/fiveMinuteStats/intro_to_em.html

Homework #7: Single-nucleotide Genotype Caller

Implement a standard binomial mixture model described in Lecture 2.

- Learn the parameters and infer the genotypes
- Annotate the mutation status for a set of genomic loci.
- Expected outputs for each question will be provided so that you can check your code.
- RStudio Markdown and Python Jupyter Notebook templates provided.

Due: May 19th, 2023